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(54) Title: **COMPOSITIONS WITH A BIOSTIMULATING ACTIVITY**

(57) Abstract: A description follows of compositions with a biostimulating activity comprising at least two components selected from a thiazolidine-4-carboxylic acid, an N-acyl derivative of thiazolidine-4-carboxylic acid and/or an amino acid component consisting of one or more essential amino acid.



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## COMPOSITIONS WITH A BIOSTIMULATING ACTIVITY

The present invention relates to compositions with a biostimulating activity and their agronomic use.

10        It has been known for some time that R-(-)-thiazolidine-4-carboxylic acid [CAS 34592-47-7] (hereafter also indicated as ATC) and some of its derivatives, in particular, N-acetylthiazolidine-4-carboxylic acid [CAS 5025-82-1] (hereafter also indicated as AATC) are  
15        used singly as components of compositions with a biostimulating activity to re-establish the correct physiological equilibrium of plants subject to various kinds of abiotic or biotic stress.

         Different kinds of stress can be caused, for example,  
20        ple, by cold, heat, drought, or by exogenous chemical agents such as herbicidal compounds, fungicidal compounds or insecticides, or other kinds of stress can be caused by specific development phases of the vegetables themselves which create particular energy consumption, such  
25        as allegation, blossoming, fruit enlargement, etc.

The Applicant has surprisingly found that, by applying compositions with a biostimulating activity consisting of thiazolidine-4-carboxylic acid mixed with N-acyl derivatives of said thiazolidine-4-carboxylic acid, there  
5 is a synergic activity between these compounds.

The Applicant has also surprisingly found that, by applying compositions with a biostimulating activity consisting of thiazolidine-4-carboxylic acid and/or an N-acyl derivative of thiazolidine-4-carboxylic acid and/or  
10 an amino acid component consisting of one or more essential amino acids, there is an amplification of the biostimulating effect of the above composition, or a synergic activity when said amino acid component proves to have a biostimulating activity.

15 An object of the present invention therefore relates to compositions comprising at least two components selected from thiazolidine-4-carboxylic acid, an N-acyl derivative of thiazolidine-4-carboxylic acid and an amino acid component consisting of one or more essential amino  
20 acids.

A further object of the present invention relates to the use of compositions comprising at least two components selected from thiazolidine-4-carboxylic acid, an N-acyl derivative of thiazolidine-4-carboxylic acid and an  
25 amino acid component consisting of one or more essential

amino acids, as biostimulants in the agronomic field.

In particular, the N-acyl derivative of thiazolidine-4-carboxylic acid is preferably selected from N-acetyl-thiazolidine-4-carboxylic acid, N-propanoyl-thiazolidine-4-carboxylic acid or N-butanoyl-thiazolidine-4-carboxylic acid.

Preferred compositions according to the present invention comprise thiazolidine-4-carboxylic acid and N-acetyl-thiazolidine-4-carboxylic acid or thiazolidine-4-carboxylic acid and an amino acid component consisting of one or more essential amino acids or N-acetyl-thiazolidine-4-carboxylic acid and an amino acid component consisting of one or more essential amino acids.

A further preferred composition according to the present invention comprises thiazolidine-4-carboxylic acid and N-acetyl-thiazolidine-4-carboxylic acid and an amino acid component consisting of one or more essential amino acids.

The N-acyl derivatives of thiazolidine-4-carboxylic acid can be present in acid form or in salified form such as salts of sodium, potassium, magnesium, copper, iron, manganese, calcium, or of another inorganic cation.

Furthermore, the N-acyl derivatives of thiazolidine-4-carboxylic acid can be present in salified form such as ammonium salts, or salified with an organic amine, such

as triethylamine, cyclohexylamine, trimethylamine, tri-ethanolamine, or with an amine group of a basic amino acid such as Glutamic acid, or ornithine.

The amino acid component consisting of one or more  
5 essential amino acids is preferably selected from proline and/or glutamic acid and/or glutamine. In particular the proline and/or glutamic acid and/or glutamine can be in purified form or as complex mixtures.

The amino acid components can consist of amino acids  
10 in purified form or consist of complex mixtures deriving from partial or total hydrolysis of biological matrixes containing protein fractions.

These amino acids or mixtures of essential amino acids can therefore be free amino acids or proteinic amino  
15 acids, which, within the context of the present invention, are considered as being precursors of free amino acids.

Said proteinic amino acids can consequently be incorporated in dipeptides, tripeptides or oligopeptides or  
20 peptides with different molecular weights.

The amino acid component according to the present invention can therefore consist of hydrolyzed proteinic products obtained from natural substrates.

These hydrolyzed products can have varying degrees  
25 of hydrolysis and will therefore have free amino acids

and/or dipeptides, tripeptides or oligopeptides with varying molecular weights. Said hydrolyzed proteinic products preferably contain at least 5% of free amino acids with respect to the total amino acid content, and  
5 will preferably have an overall minimum content of total amino acids (free or proteinic) of 25% w/w.

The hydrolyzed proteinic products are preferably hydrolyzed proteinic products of bovine epithelium or vegetable matrixes.

10 Said hydrolyzed product will preferably contain essential amino acids, such as proline, glutamic acid or glutamate, or glutamine and can have its own biostimulating activity on numerous crops of economic interest.

The use of the composition according to the present  
15 invention is particularly advantageous specifically as a result of the synergic effect observed in the application of the compositions with a biostimulating activity comprising at least two components selected from thiazolidine-4-carboxylic acid, an N-acyl derivative of thiazolidine-4-carboxylic acid and an amino acid component  
20 consisting of one or more essential amino acids, when said amino acid component proves to have its own biostimulating activity.

A further advantage consists in the amplification of  
25 the biostimulating effect observed in the application of

the compositions with a biostimulating activity comprising at least two components selected from thiazolidine-4-carboxylic acid, an N-acyl derivative of thiazolidine-4-carboxylic acid and an amino acid component consisting of  
5 one or more essential amino acids, when said amino acid component does not have its own biostimulating activity.

In agronomic practice, the compositions object of the present invention can conveniently contain additives, dispersants, wetting agents and any other product known  
10 in the art for favouring the application of active substances to vegetables.

An object of the present invention therefore also relates to a method for the biostimulation of agricultural crops by the application of the compositions according to the present invention, alone or with other  
15 compounds having the same or a different activity.

The application of the compositions object of the present invention is effected on the whole plant or a part of it, by application on the aerial part and/or on  
20 the hypogeum, for example by dispersion or spraying, on the leaves or via the roots.

Said thiazolidine-4-carboxylic and N-acyl-thiazolidine-4-carboxylic acids and the amino acid components are generally commercial products easily available  
25 on the market. The hydrolyzed proteinic product from bo-

vine epithelium, sold in Italy and in many other countries in the world under the trade mark of Siapton® and the hydrolyzed product based on vegetables (soybean) called Fertimin® are examples of a complex amino acid  
5 mixture.

The following examples are provided for merely illustrative purpose and in no way intend to limit the scope of the present invention. In the following examples the synergic effect of the components of the mixture is  
10 revealed by comparing the experimental data with the theoretical efficacy of the mixture, object of the invention, calculated according to the Limpel formula ("Pesticide Science" (1987), vol. 19, pages 309-315):

$$E = x + y - (xy/100)$$

15 wherein :

- E is the biological activity expected, in the absence of synergic effects, from a mixture obtained by mixing x g of compound X with y g of compound Y;
- x is the activity of the compound X when used alone  
20 at a dose of x g;
- y is the activity of the compound Y when used alone at a dose of y g.

When the biological activity experimentally found is higher than the value of E, said activity should be con-  
25 sidered as a synergic effect.



EXAMPLE 1

Preparation of compound with a biostimulating activity for the subsequent experiments.

An amount of 50 g of each compound under examination  
5 (thiazolidine-4-carboxylic acid (compound I) and N-acyl-  
thiazolidine-4-carboxylic acid (compound II)) were dis-  
persed in water (800 cc) and vigorously stirred. An ex-  
cess of 5%, with respect to the stoichiometric quantity,  
of sodium bicarbonate, was then added in portions. 150 g  
10 of a non-ionic surface-active agent were added to favour  
application to the leaves. The whole mixture was brought  
to a volume of 1 liter by the addition of water, in order  
to obtain solutions of each compound with a concentration  
of 50 g/liter.

15 EXAMPLE 2

Assessment of the biostimulating activity of compound  
(I), compound (II) and their synergic mixture.

Seedlings of courgettes cv. Diamone, sown and culti-  
vated in a hot greenhouse, were transplanted in a field  
20 (May 11, Leggiuno, (Varese) - Italy).

The theses were randomly arranged in lots with four  
repetitions for each thesis. Each lot consisted of six  
plants in rows situated at a distance of 1 meter from  
each other, and at a distance of 1 meter from each other  
25 in the same row.

Weeding was performed before the transplant, using 1 kg/ha of trifluralin (volume 550 l/ha).

At the moment transplanting, the seedlings have a common degree of vegetative development.

5 In order to minimize transplant stress, the seedlings planted were treated respectively with:

Thesis A : with compound (I) (ATC) at a dose of 5 g/hl;

10 Thesis B : with compound (I) (ATC) at a dose of 10 g/hl;

Thesis C : with compound (II) (AATC) at a dose of 5 g/hl;

Thesis D : with compound (II) (AATC) at a dose of 10 g/hl;

15 Thesis E : with compound (I) (ATC) at a dose of 5 g/hl in a mixture with compound (II) (AATC) at a dose of 5 g/hl;

Thesis F : no biostimulating treatment.

20 Said treatment was subsequently repeated every 10 days, in a preventive manner, in order to oppose any possible thermal or water stress or stress due to vegetative development.

Harvesting was effected from June 11 to August 27, and the yield increases indicated in Table 1, were registered  
25 in the treated theses (A, B, C, D, E), with respect to

the non-treated Thesis F. The yields are obtained from the average amount of fruit obtained in each lot during the above-mentioned harvesting period (June 11 - August 27).

5      Table 1

	T H E S I S					
	A (5 g ATC/h)	B (10 g ATC/h)	C (5 g AATC/h)	D (10 g AATC/h)	E (5 g AATC/h + 5 g ATC/h)	F (not treated)
10      Fruit per lot	100	105	102	109	115	97
Increase (%)	+ 3.1 %	+ 8.2 %	+ 5.2 %	+ 12.4 %	+ 18.6 %	---

From the experimental results obtained, the expected activity (E) for the mixture of 5 g of compound I plus 5 g of compound II (Thesis E), calculated by applying to said experimental results the above-mentioned Limpel formula, is:

$$E = (3.1+8.2) - (3.1 \cdot 8.2)/100 = 11\%$$

The ratio between the experimental data and the expected activity proves to be higher than 1, indicating a synergy of effects:

$$18.6/11 = 1.69$$

### EXAMPLE 3

Determination of the amplification of the biostimulating effect obtained by applying compound (I) in the presence

of a solution of proline.

Seedlings of peppers cv. Pathos were transplanted in a tunnel (April 16, AT).

The lots were distributed according to the random block scheme with 5 repetitions per thesis. Each repetition consisted of 15 plants.

At the moment of transplanting, the seedlings have a common degree of vegetative development.

In order to minimize transplant stress, the seedlings planted were treated respectively with:

Thesis A : with compound (I) (ATC) at a dose of 10 g/hl;

Thesis B : with compound (I) (ATC) at a dose of 10 g/hl, an aqueous solution of proline at a dose of 100 g/hl;

Thesis C : with compound (II) (AATC) at a dose of 10 g/hl;

Thesis D : with compound (II) (AATC) at a dose of 10 g/hl, an aqueous solution of proline at a dose of 100 g/hl;

Thesis E : no biostimulating treatment.

Said treatment was subsequently repeated every 10 days, in a preventive manner, in order to oppose any possible thermal or water stress or stress due to vegetative development.

Harvesting was effected from July 29 for the following 30 days, and a production increase was registered in theses B and D, with respect to theses A and C, as indicated in Table 2. The yields are obtained from the average production obtained during the above harvesting period (July 29 - August 29).

Table 2

	T H E S I S				
	A (10 g ATC/hl)	B (10 g ATC/hl + 100 g Proline/hl)	C (10 g AATC/hl)	D (10 g AATC/hl + 100 g Pro- line/hl)	E (not treated)
Berries kg per lot	25.80	27.80	26.40	28.20	24.70
Increase (%)	+ 4.5 %	+12.6 %	+ 6.9 %	+ 17.0 %	—

As it is known that the proline solutions do not have their own biostimulating activity, this amplification is attributed to a synergic interaction of the proline itself on the biostimulating activity of compounds (I) and (II).

EXAMPLE 4

Determination of the synergic effect obtained in the application of a mixture based on compound (I) and compound (II) to which a mixture is added, containing essential amino acids, consisting of a hydrolyzed proteinic product

obtained from bovine epithelium, with a known biostimulating activity.

Tomato seedlings cv. Arletta, sown and cultivated in a greenhouse, were transplanted in a field (May 11, Leggiuno (VA)- Italy), after weeding with trifluralin (1 kg/ha).

The theses were arranged in random blocks. Each lot consisted of 10 plants at a distance of 50 cm from each other in rows 80 cm apart.

10       At the moment of transplanting, the seedlings have a common degree of vegetative development.

In order to minimize transplant stress, the seedlings planted were treated respectively with:

Thesis A : with compound (I) (ATC) at a dose of 5 g/hl  
15 mixed with compound (II) (AATC) at a dose of 5 g/hl;

Thesis B : with a hydrolyzed proteinic product of an animal origin (compound III), at a dose of 500 g/hl;

Thesis C : with compound (I) (ATC) at a dose of 5 g/hl  
mixed with compound (II) (AATC) at a dose of 5 g/hl mixed  
20 with a hydrolysed proteinic product (compound III) at a dose of 500 g/hl;

Thesis D : no biostimulating treatment.

Said treatment was subsequently repeated every 15 days, in a preventive manner, in order to oppose any possible thermal or water stress or stress due to vegetative  
25

development.

In particular, the compound (III) used in this test, for the sole and non-limiting purpose of illustrating the behaviour of a hydrolyzed proteinic product, is a sample  
5 of Siapton®, a hydrolyzed product of bovine epithelium, characterized by an overall content of about 700 g of amino acids (free and proteinic) per liter of solution, of which about 13% w/w consists of proline, about 12% of glutamic acid, about 9.5% of 4-hydroxyproline.

10 Harvesting was effected from July 16 to September 9, and the yield increases indicated in Table 3, were registered in the theses treated (A, B, C) with respect to the non-treated Theses D. The yields are obtained from the average amount and average weight of the fruit obtained  
15 per lot, during the above harvesting period (July 16 - September 9).

Table 3

	T H E S I S				
	A (5 g AATC/hl + 5 g ATC/hl)	B Compound III (XX g/hl)	C (5g AATC/hl + 5g ATC/hl) + Compound III (500 g/hl)	C Expected Activity according to Limpel	D (not treated)
Berries per lot	178	173	193		161
Increase (%)	+10.6 %	+7.5 %	+ 19.9 %	+ 18.0 %	---
Berries kg/lot	22.10	21.40	25.10		18.60
25 Increase (%)	+ 18.8 %	+ 15.1 %	+ 34.9 %	+ 33.8 %	---

The expected values applying the Limpel formula are lower than those obtained experimentally showing a synergy of effects.

#### EXAMPLE 5

5 Determination of the synergic effect obtained in the application of a mixture based on compound (I) and compound (II) to which a mixture is added, containing essential amino acids, consisting of a hydrolyzed proteinic product of a vegetable origin (compound IV).

10 Aubergine seedlings cv. Violetta Lunga, sown and cultivated in a hot greenhouse, were transplanted in the middle of a field on May 14 (Leggiuno (VA)- Italy), after weeding with trifluralin (1 kg/ha).

The theses were distributed according to 5 random  
15 blocks. Each lot consisted of 8 plants at a distance of 40 cm from each other on each row. The distance between the rows was 1 m.

At the moment of transplanting, the seedlings have a common degree of vegetative development.

20 In order to minimize transplant stress, the seedlings planted were treated respectively with:

Thesis A : with compound (I) (ATC) at a dose of 5 g/hl mixed with compound (II) (AATC) at a dose of 5 g/hl;

Thesis B : with a hydrolyzed proteinic product (com-  
25 pound IV) with a minimum content of proline of 1.5%



(meaning overall free or proteinic proline) at a dose of 900 g/hl;

Thesis C : with compound (I) (ATC) at a dose of 5 g/hl mixed with compound (II) (AATC) at a dose of 5 g/hl mixed  
5 with a hydrolysed proteinic product (compound IV) at a dose of 600 g/hl;

Thesis D : no biostimulating treatment.

Said treatment was subsequently repeated every 10 days, in a preventive manner, in order to oppose any possible thermal or water stress or stress due to vegetative  
10 development.

In particular, the compound (IV) used in this test, for the sole and non-limiting purpose of illustrating the behaviour of a hydrolyzed proteinic product, is a hydro-  
15 lyzed proteinic product obtained from soybean and characterized by a minimum content of proline of about 1.5%, a minimum content of glutamic acid of about 5% and the total absence of 4-hydroxyproline.

Harvesting was effected from August 1 to September  
20 12, and the yield increases indicated in Table 4, were registered in the theses treated (A, B, C) with respect to the non-treated Theses D. The yields are obtained from the average amount of fruit obtained for each lot, during the above harvesting period (August 1 - September 12).

Table 4

	T H E S I S				
	A (5 g AATC/hl + 5 g ATC/hl)	B Compound IV (600 g/hl)	C (5g AATC/hl) + 5 g ATC/hl) + Com- pound IV (600 g/ha)	C Expected Activity ac- cording to Limpel	D (not treated)
Berries per lot	28.1	26.7	30.4		25.8
Increase (%)	+ 8.9 %	+ 3.5 %	+ 17.8 %	+ 12.4 %	---

10        The expected values applying the Limpel formula are  
lower than those obtained experimentally showing a syn-  
ergy of effects.

CLAIMS

1. Compositions comprising at least two components selected from thiazolidine-4-carboxylic acid, an N-acyl derivative of thiazolidine-4-carboxylic acid and an amino acid component consisting of one or more essential amino acids.
2. The compositions according to claim 1, characterized in that the N-acyl derivative of thiazolidine-4-carboxylic acid is selected from N-acetyl-thiazolidine-4-carboxylic acid, N-propanoyl-thiazolidine-4-carboxylic acid or N-butanoyl-thiazolidine-4-carboxylic acid.
3. The compositions according to claim 1, characterized in that they comprise thiazolidine-4-carboxylic acid and N-acetyl-thiazolidine-4-carboxylic acid.
4. The compositions according to claim 1, characterized in that they comprise thiazolidine-4-carboxylic acid and an amino acid component consisting of one or more essential amino acids.
5. The compositions according to claim 1, characterized in that they comprise N-acetyl-thiazolidine-4-carboxylic acid and an amino acid component consisting of one or more essential amino acids.
6. The compositions according to claim 1, characterized in that they comprise thiazolidine-4-carboxylic acid, N-acetyl-thiazolidine-4-carboxylic acid and an amino acid

component consisting of one or more essential amino acids.

7. The compositions according to claim 1, characterized in that the N-acyl derivatives of thiazolidine-4-carboxylic acid are present in acid form or in salified form.

8. The compositions according to claim 7, characterized in that the N-acyl derivatives of thiazolidine-4-carboxylic acid are present in salified form as salts of sodium, potassium, magnesium, copper, iron, manganese, calcium, or another inorganic cation.

9. The compositions according to claim 7, characterized in that the N-acyl derivatives of thiazolidine-4-carboxylic acid are present in salified form as ammonium salts, or salified with an organic amine such as triethylamine, cyclohexylamine, trimethylamine, triethanolamine, or with the amine group of a basic amino acid such as glutamic acid or ornithine.

10. The compositions according to claim 1, characterized in that the amino acid component consisting of one or more essential amino acids is selected from proline and/or glutamic acid and/or glutamine.

11. The compositions according to claim 10, characterized in that proline and/or glutamic acid and/or glutamine are in purified form or are complex mixtures.

12. The compositions according to claim 1, characterized in that the essential amino acids are in purified form or consist of complex mixtures deriving from partial or total hydrolyses of biological matrixes containing proteinic fractions.

13. The compositions according to claim 1, characterized in that the amino acids or mixtures of essential amino acids consist of free amino acids or proteinic amino acids, precursors of free amino acids.

10 14. The compositions according to claim 1, characterized in that the amino acid component consists of hydrolyzed proteinic products obtained from natural substrates.

15 15. The compositions according to claim 14, characterized in that the hydrolyzed proteinic products contain at least 5% of free amino acids with respect to the total content of amino acids.

16. The compositions according to claim 14, characterized in that the hydrolyzed proteinic products have an overall minimum content of total amino acids (free or proteinic) of 25% w/w.

17. The compositions according to claim 14, characterized in that the hydrolyzed proteinic products are hydrolyzed proteinic products of bovine epithelium.

18. The compositions according to claim 14, characterized in that the hydrolyzed proteinic products are hydro-

lyzed proteinic products of vegetable matrixes.

19. The compositions according to any of the previous claims, characterized in that they comprise further additives, dispersants and/or wetting agents.

5 20. A method for biostimulating agricultural crops by the application of the compositions according to one of the claims 1-19, alone or with other compounds having an analogous or different activity.

21. The method according to claim 20, characterized in  
10 that the application takes place on the whole plant or on a part of the plant, by means of applications on the aerial part and/or on the hypogeum, for example, by dispersion or spraying, either on the leaves or via the roots.

22. Use of the compositions according to any of the  
15 claims from 1 to 19, as biostimulants in the agronomic field.

## INTERNATIONAL SEARCH REPORT

Internal Application No  
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A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A01N43/78

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; YAMAMURA, SABURO: "Growth promoter composition for sugar beets and sugarcane" retrieved from STN Database accession no. 105:166894 XP002243348 abstract &amp; JP 61 100504 A (HOKKO CHEMICAL INDUSTRY CO., LTD., JAPAN) 19 May 1986 (1986-05-19) --- -/--</p>	1-22

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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Internat Application No  
PCT/EP 03/03193

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CABA 'Online! LOVATO, L. A.: "Biostimulant for an irrigated rice crop Bioestimulante para a cultura do arroz irrigado" retrieved from STN Database accession no. 81:44710 XP002243349 abstract &amp; ANAIS, VII REUNIAO DA CULTURA DO ARROZ, RIO GRANDE DO SUL, PP. 157-158. PUBLISHER: EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA. PELOTAS,  Instituto Rio Grandense do Arroz, Porto Alegre 90.000, Rio Grande do Sul, Brazil. ---</p>	1-22
X	<p>EP 0 078 514 A (MONTEDISON SPA) 11 May 1983 (1983-05-11). the whole document -----</p>	1-22



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PCT/EP 03/03193

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